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Inhibin: Actions and Signalling

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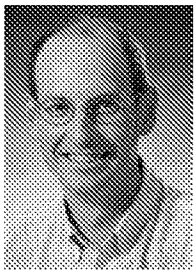

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Inhibin is best known as a reproductive hormone that inhibits release of follicle-stimulating hormone (FSH) from the pituitary gland. Over the last decade or so a number of other biological roles have emerged, putting inhibin more in the class of a growth factor. Despite this, little is known of the signalling pathways for this protein. This minireview summarises the pertinent aspects of inhibin biology and focuses on four potential signalling mechanisms through which inhibin might influence cellular function, namely subunit availability, receptor assembly, co-receptors and signalling through inhibin-specific signalling pathways.

Keywords: Transforming growth factor-β; Inhibin; Activin; Betaglycan

INTRODUCTION

Unlike many growth factors, the instigation of "inhibin" as an entity can be placed as far back as the 1930s, when D. Roy McCullagh postulated of a non-steroidal factor produced by the gonad that influenced the regulation of hormones from the pituitary. However, it was not until 1985 that inhibin was actually purified and characterised (Ling *et al.*, 1985; Robertson *et al.*, 1985). Since then, there has been a plethora of findings investigating the actions, effects and sources of inhibin, and recent studies have begun to shed light on how inhibin signals. This minireview will focus on the potential mechanisms by which this fascinating protein signals, but first it is pertinent to consider the structure of inhibin and to what roles in various tissues it has been ascribed.

BASIC STRUCTURE AND ACTIONS

It is now recognised, based largely on structural homology, that inhibin is a member of the transforming growth factor-β (TGF-β) superfamily of growth factors. The inhibins, of which there are actually two forms known, consist of an α-subunit dimerised to a β-subunit; they share β-subunits with that of the activin family of factors (Phillips, 2003). Thus, inhibin A ($\alpha-\beta_A$) and inhibin B ($\alpha-\beta_B$) are made up of the corresponding dimers. Interestingly, inhibins consisting of the other β subunits (e.g. $\alpha-\beta_C$, inhibin C) do not appear to be formed (Mellor *et al.*, 2000). The mature inhibin dimer has a molecular weight of around 32 kDa, although larger molecular weight forms consisting of unprocessed pro-forms and variant glyco-forms are known to exist in serum and be produced by various cell types

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(Robertson *et al.*, 2001). Indeed, the early and widely utilised assays for inhibin recognised these alternate forms in addition to the mature dimers. While informative in defining the biology of inhibin, several findings thrown up by these assays were somewhat perplexing. For instance, inhibin levels were postulated to be negatively correlated with the reproductive hormone, follicle-stimulating hormone (FSH), in line with McCullagh's original hypothesis. Formal evidence for this proved elusive until the development of ELISA-based assays that were specific for either inhibin A or B. When these assays came on line, they provided the intriguing finding that while both sexes had detectable production of both inhibins, by far and away the dominant and important inhibin in human males was inhibin B (Illingworth *et al.*, 1996). A caveat to the above is that in another species, the sheep, inhibin A appears to be the dominant inhibin in males (McNeilly *et al.*, 2002). The cell types in the testis responsible for inhibin production are the Sertoli cells within the seminiferous epithelium and a minor component arising from the Leydig cells in the interstitial spaces. A strong relationship between Sertoli cell number and circulating inhibin levels has been demonstrated, suggesting that inhibin may be a useful clinical marker of testicular function (Tong *et al.*, 2003).

As initially predicted, a clear action of inhibin is to suppress FSH secretion from the pituitary. This has been convincingly demonstrated in adult males using human recombinant inhibin (Tilbrook *et al.*, 1993). While a classical feedback hormone action is likely to account for most of this effect of inhibin, a local paracrine role in the pituitary cannot be discounted. This is pertinent given the demonstration that the inhibin co-receptor, betaglycan, is produced and dynamically regulated according to the stage of the oestrous cycle in rats (Chapman and Woodruff, 2003).

In terms of the female, inhibin may play a role as a paracrine factor in the ovary, with the two inhibins dominant in the circulation at different stages of the menstrual cycle (Groome *et al.*, 1996). Inhibin A is likely to be the product of the ovulatory follicle and remains so as it transforms to the corpus luteum, whereas inhibin B is mainly a reflection of the cohort of recruited follicles as they progress towards selection. How inhibin functions within the ovary has been the subject of recent array profiling. While a number of candidate genes were identified in ovaries from inhibin α knockout mice, the function of many of them is unclear or they appear to be involved in cell metabolism (Burns *et al.*, 2003). This is consistent with studies showing normal reproductive function in mice deficient in the inhibin binding protein, InhBP/p120 (Bernard *et al.*, 2003).

Much of the function(s) of inhibin has been inferred from the use of specific inhibin assays to gain information about particular organs, physiological conditions, or disease states, summarised in Fig. 1. For instance, a role in pregnancy was raised when it was documented that inhibin levels changed dynamically in the circulation of pregnant women (McLachlan *et al.*, 1987; Muttukrishna *et al.*, 1995). The pregnant mother, the placenta and the fetus itself have been implicated as sources of inhibin in pregnancy. Subsequent to this, perturbations in inhibin have been noted in the detection of early pregnancy viability, corpus luteum function and in abnormalities such as Down's syndrome pregnancies and in pre-eclampsia (Tong *et al.*, 2003).

The concept that inhibin may in fact be a tumour suppressor gene arose initially from mice with the inhibin α-subunit gene knocked out, where both sexes developed tumours in the ovaries and testes (Matzuk *et al.*, 1992). When the animals were gonadectomised, tumours then developed in the adrenal

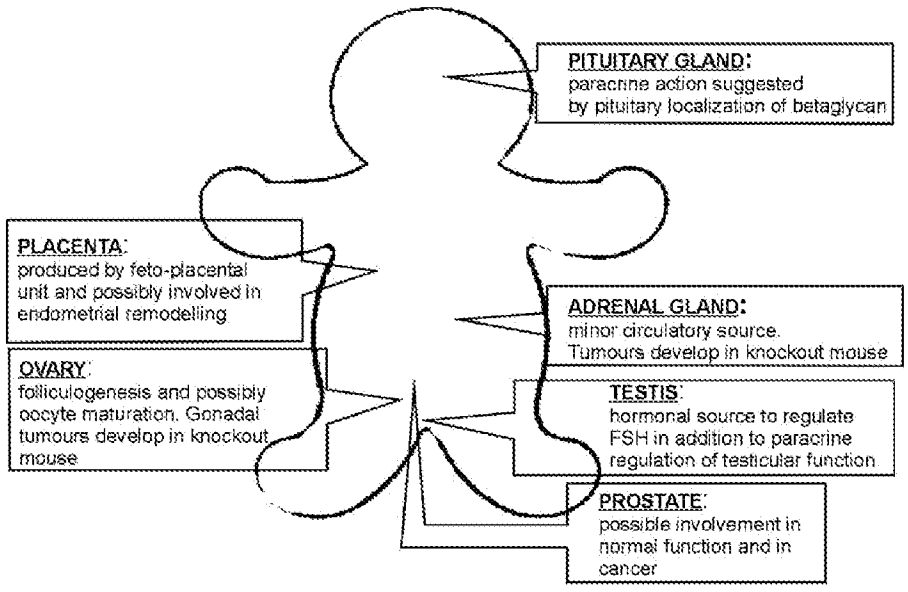

FIGURE 1 Body organs in which the inhibins are known to have a function and putative roles. Note this is not an inclusive list.

glands. Subsequently, a number of studies have linked inhibin with carcinogenesis in several situations, although the precise mechanism(s) by which inhibin may be acting as a tumour-suppressor gene has yet to be resolved (Risbridger *et al.*, 2001).

POTENTIAL SIGNALLING PATHWAYS

In comparison to what is known about signal transduction by other members of the TGF-β superfamily, the mechanism of inhibin signalling largely remains a mystery. This is due, in part, to the failure to identify and clone an inhibin receptor, to characterise its signal transduction pathway, or to identify inhibin-regulated target genes. It is possible that a true inhibin receptor does not exist, and that inhibin's actions are mediated not by an independent signalling mechanism, but rather through the modulation of activin signal transduction. Indeed, inhibin action has been largely characterised in terms of its ability to antagonise activin action; the best known example is the inhibition of activin-stimulated FSH production by inhibin in the pituitary (Woodruff and Mather, 1995). In addition, inhibin also abrogates local activin actions in the gonads, where it antagonises inhibition of testosterone production by activin in cultured rat testicular cells and thecal cells, and opposes activin-stimulated 3β-hydroxysteroid dehydrogenase expression in cultured porcine Leydig cells (Lin *et al.*, 1989; Lejeune *et al.*, 1997). However, the molecular basis of the inhibitory effect of inhibin on activin actions is not completely understood. To date, there are four proposed mechanisms of inhibin signal transduction.

Ligand Assembly

Because inhibin and activin dimers are assembled using a common pool of β-subunits, the production of these ligands depends upon the availability of the α-subunit. In the gonads, α-subunit expression is greater than that of the β-subunits, and inhibin dimer production exceeds that of activin (Woodruff *et al.*, 1988; Magoffin and Jakimiuk, 1997). Therefore, paracrine activin signalling in the ovary would be predicted to be abrogated by inhibin simply because less activin dimer is assembled.

Heteromeric Receptor Complex Assembly

Activin binds to activin type II receptors, either ActRIIA or ActRIIB, and then recruits and phosphorylates a type I receptor (activin receptor-like kinase-4 or ALK4) into an active heteromeric receptor complex. Inhibin also binds to ActRIIA and ActRIIB via its β-subunit although with lower affinity than does activin (Mathews and Vale, 1991; Xu *et al.*, 1995; Martens *et al.*, 1997), but does not stimulate recruitment or phosphorylation of ALK4 (Lebrun and Vale, 1997; Martens *et al.*, 1997). Thus, when the concentration of inhibin exceeds that of activin, inhibin can bind the activin type II receptor and functionally antagonise activin signalling, by competing for activin binding to its own receptor or by interfering with additional activin receptor complex assembly (Dyson and Gurdon, 1998). This functional antagonism by inhibin is observed in the reversal of growth inhibition by activin in the HepG2 liver cell line (Xu *et al.*, 1995), as well as in the inhibition of activin-stimulated junB expression in Chinese hamster ovary cells by inhibin (Martens *et al.*, 1997).

Inhibin-binding Co-receptors

In the KAR6 erythroleukemia cell line, inhibin is unable to antagonise activin-stimulated cell differentiation, cell proliferation, activin receptor complex formation and reporter gene activity, even at far higher concentrations than activin (Lebrun and Vale, 1997). This is also presumably true for other cell lines, such as the mouse B-cell line, MPC-11 (Phillips *et al.*, 1999). Additionally, inhibin does not block activin-stimulated apoptosis in hepatocytes (Schwall *et al.*, 1993), nor does it affect activin's suppression of adrenocorticotropin hormone (ACTH) from AtT20 corticotrope cells (Bilezikjian *et al.*, 1991). Conversely, inhibin is able to antagonise activin-stimulated FSH secretion and FSH-β expression in cultured primary pituitary cells at low or equimolar concentrations (Carroll *et al.*, 1989; Rivier and Vale, 1991; Weiss *et al.*, 1993). Thus, it was proposed that an inhibin-binding receptor was present in pituitary cells that was absent in other systems, necessary to mediate inhibin antagonism of activin in inhibin target tissues.

In the ensuing search for this protein, it was discovered that inhibin A binds the TGF-β type III receptor, betaglycan (Lewis *et al.*, 2000). Betaglycan is a membrane-bound proteoglycan that binds TGF-β2 and interacts with the TGF-β heteromeric receptor complex to enhance signalling (López-Casillas *et al.*, 1993). Because betaglycan protein does not have intrinsic kinase activity, it is referred to as a co-receptor or accessory protein. Introduction of betaglycan into AtT20 cells confers inhibin sensitivity, allowing even low concentrations of inhibin to antagonise activin-stimulated ACTH secretion (Lewis *et al.*, 2000). Inhibin-bound betaglycan interacts with the activin type II receptors and it is hypothesised that it either inhibits activin binding or prevents the formation of activin heteromeric receptor complexes (see, Fig. 2, Lewis *et al.*, 2000; Esparza-Lopez *et al.*, 2001; Chapman *et al.*, 2002). Further studies of the inhibin-binding properties of betaglycan suggest that its function may depend on the available form of activin type II receptor and inhibin isoform (Esparza-Lopez *et al.*, 2001; Chapman *et al.*, 2002). Finally, betaglycan has been shown to be expressed in inhibin target tissues (Drummond *et al.*, 2002; MacConell *et al.*, 2002), and is expressed on the surface of pituitary gonadotrophs when FSH levels fall following the primary and secondary

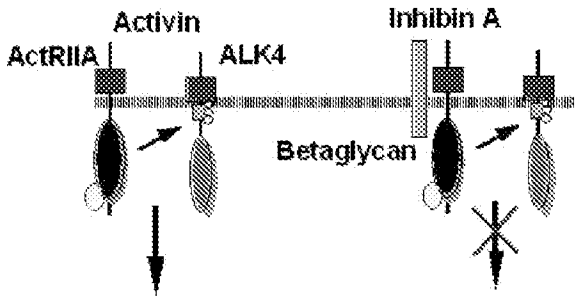

FIGURE 2 One of the mechanisms proposed for inhibin action is to utilise betaglycan to occupy the ActRII binding subunit of the activin receptor complex. In doing so, inhibin disengages the activin pathway at the first step in the signal transduction cascade.

gonadotrophin surges in the rat oestrous cycle (Chapman and Woodruff, 2003). A recent report described that mice null for betaglycan develop lethal defects in heart and liver development by midgestation, and cell lines derived from the betaglycan-null embryos exhibit reduced sensitivity to TGF-β2 (Stenvers *et al.*, 2003). Confirmation of the role of betaglycan in mediating inhibin antagonism of activin action *in vivo*, and particularly its role in the postnatal reproductive axis, awaits the creation of betaglycan overexpression and inducible knockout animal models.

Surprisingly, two studies have demonstrated that inhibin and betaglycan may be general modulators of TGF-β superfamily signalling. A recent study demonstrated that inhibin A antagonism of two activin-responsive reporter constructs could be rescued by TGF-β, which competes with inhibin for binding to betaglycan (Ethier *et al.*, 2002). In addition, betaglycan has recently been implicated in the mediation of inhibin antagonism of BMP signalling, through both activin type II receptors and the BMP type II receptor (Wiater and Vale, 2003).

Another accessory protein, InhBP/p120, was purified from the bovine pituitary gland and found to permit inhibin B antagonism of activin-stimulated p3TP-luc expression in the TSA cell line (Chong *et al.*, 2000; Chapman and Woodruff, 2001). However, InhBP/p120 does not bind either inhibin isoform (Chapman *et al.*, 2002), and InhBP/p120 null mice exhibit no reproductive abnormalities (Bernard *et al.*, 2003).

Independent Inhibin Receptor

The fourth inhibin signalling model returns to the idea that inhibin may bind to its own inhibin receptor, which then transduces a signal and directs inhibin-regulated cellular events. Supporting this hypothesis is the finding that inhibin action is not always defined by its ability to antagonise activin action. For example, when all pituitary activin is neutralised by an activin-specific antibody, inhibin is still able to block pituitary FSH secretion (Murata *et al.*, 1996). Also, inhibin A stimulates the production and secretion of androgens by cultured thecal cells, upregulates expression of the steroidogenic enzyme, P450c17, and that of the LH receptor in primary cultures of porcine Leydig cells, and promotes primate oocyte maturation *in vitro* (Lin *et al.*, 1989; Hillier *et al.*, 1991; Miro and Hillier, 1992; Alak *et al.*, 1996; Lejeune *et al.*, 1997). A recent study demonstrated that loss of inhibin in female rats, either by ovariectomy or by immuno-neutralisation, led to a increase in follistatin transcription and mRNA stability (Prendergast *et al.*, 2003). Furthermore, administration of exogenous inhibin A reduced follistatin transcription. In each of these cases, inhibin action may require the presence of an independent inhibin receptor to transduce an inhibin signal and effect downstream cellular events.

The independent inhibin receptor model is also supported by the discovery of inhibin-specific binding sites in several tissues and cell lines. Iodinated inhibin A, but not iodinated activin A, binds to rat Leydig cells (Krummen *et al.*, 1994), and the adrenal, spleen, and bone marrow bind iodinated inhibin A at much higher levels than they bind iodinated activin A (Woodruff *et al.*, 1993). Inhibin binding proteins have also been identified in bovine pituitaries and human erythroleukemia cells (Lebrun and Vale, 1997; Chong *et al.*, 2000). Gonadal tumours derived from inhibin α-subunit knockout mice bind iodinated inhibin A, and inhibin binding is not displaced by excess unlabelled activin (Draper *et al.*, 1998). Furthermore, the predicted sizes of the inhibin binding proteins in the inhibin α-subunit knockout tumours (40, 54, 84, and 98 kDa) do not correspond to either betaglycan (>110 kDa) or InhBP/p120 (~140 kDa) (Draper *et al.*, 1998). In porcine pituitary cells, inhibin binds to both high (280–310 pM) and low affinity (3.9–5.3 nM) sites, which may correspond to either ActRIIA alone (6.3 nM) or with betaglycan (200 pM) (Hertan *et al.*, 1999; Lewis *et al.*, 2000; Farnworth *et al.*, 2001). High and low inhibin binding affinities (40–60 pM and 520–670 pM) are also observed in the testis-derived Sertoli (TM4) and Leydig (TM3) cell lines, and several inhibin A binding proteins and complexes were identified in each line by affinity labelling (Harrison *et al.*, 2001). The identity of the majority of inhibin binding proteins has not yet been discovered, and whether any of them directly mediate independent inhibin signalling events, or act as co-receptors to modulate activin signalling, will need to be determined.

It is also possible that an independent inhibin receptor will mediate inhibition of activin signal transduction. Intracellular inhibin signals may interfere with the activin signal transduction pathway at the level of the activin receptor or Smad proteins by interfering with their phosphorylation and activation. In addition, inhibin signals may stimulate inhibitory Smad6 or Smad7 expression or activity. Alternatively, inhibin signals may potentiate the internalisation or degradation of activin signalling components, including the activin receptors and Smads.

A recent study described the internalisation of ligand-bound TGF-β receptors by two distinct endocytic vesicles that either permit or block signal transduction (Di Guglielmo *et al.*, 2003). TGF-β receptors shuttled into clathrin-coated pits that contain Smad2 and the Smad anchor for receptor activation (SARA) would propagate the TGF-β signal. On the other hand, TGF-β receptors may be directed to caveolin-positive vesicles enriched in Smad7 and Smurf2, proteins which mediate receptor degradation and signal termination. Thus, shuttling of TGF-β receptor complexes into particular endocytic compartments would dictate whether a TGF-β signal is transduced. Perhaps an inhibin receptor or an inhibin-binding accessory protein, such as betaglycan, similarly determines the fate of an activin signal by selectively directing the activin receptor complex into caveolin-positive endocytic compartments.

CONCLUDING REMARKS

There is little doubt that the biology of inhibin has progressed in leaps and bounds over the last couple of decades. It is now recognised as having many more functions in addition to that envisaged by D. Roy McCullagh in the 1930s. Although a true inhibin receptor has yet to be discovered, several lines of evidence support the direct role of inhibin in the regulation of cellular processes, and the search for the mechanism underlying inhibin's physiological effects persists. Inhibin-specific binding proteins continue to be assessed and the effect of inhibin on cellular processes, either independent of activin or antagonistic to activin, continue to be dissected in order to understand better the mechanism of inhibin signal transduction. Thus, the area of inhibin biology, and particularly its mechanism of intracellular signalling, remain a fruitful area of research enquiry.

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